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JC643 U.S. PTO 10/23/00

EXPRESS MAIL CERTIFICATE

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Signature	M Pierce	**Date**	10-23-00

JC841 U.S. PTO 09/695531 10/23/00

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Atty Docket No. YAMA-008YAMA-008	YAMA-008
First Named Inventor	Thomas Yamashita
Title:	**MICROBIAL BLEND COMPOSITIONS AND METHODS FOR THEIR USE**

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

1. X Fee Transmittal Form

2. X Specification Total Pages 28
(preferred arrangement set forth below)
- Descriptive title of the invention
- Cross Reference to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. ___ Drawing(s) *(35 USC 113)* Total Sheets

4. X Oath or Declaration Total Sheets 2

a. X Newly executed (original or copy)
b. ___ Copy from a prior application (37 CFR 1.63(d) (for continuation/divisional with Box 16 completed)

i. ___ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b)

c. ___ Unsigned

Address to: Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

5. ___ Microfiche Computer Program *(Appendix)*

6. ___ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*

a. ___ Computer Readable Copy
b. ___ Paper Copy (identical to computer copy)
c. ___ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. ___ Assignment Papers (cover sheet & document(s))

8. ___ 37 CFR 3.73(b) Statement *(when there is an assignee)* ___Power of Attorney

9. ___ English Translation Document *(if applicable)*

10. X Information Disclosure Statement (IDS)/PTO-1449 X Copies of IDS Citations

11. ___ Preliminary Amendment

12. X Return Receipt Postcard (MPEP 503)
(Should be specifically itemized)

13. X Small Entity Statement(s) ___Statement filed in prior application Status still proper and desired

14. ___ Certified Copy of Priority Document(s)
(if foreign priority is claimed)

15. ___ Other:

16. If a CONTINUING APPLICATION, *check appropriate box and supply the requisite information:*
___ Continuation ___ Divisional ___ Continuation-in-part (CIP) of prior application No. _____.

UTILITY PATENT APPLICATION TRANSMITTAL
(Only for new non-provisional applications under 37 CFR 1.53(b))

17. CORRESPONDENCE ADDRESS			
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Individual Name	Bret E. Field
Registration No.	37,620
Signature	
Date	10.23.00

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FEE TRANSMITTAL FOR 2000

PTO/SB17

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT $355.00

Application No.	N/A
Filing Date	Herewith
First Named Inventor	Thomas Yamashita
Examiner Name	N/A
Group Art Unit	N/A
Attorney Docket No.	YAMA-008

METHOD OF PAYMENT

1. X The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:
Deposit Account No. 50-0815

FEE CALCULATION

1. FILING FEE

Large Fee Code	Entity Fee ($)	Small Fee Code	Entity Fee ($)	Fee Description	Fee Due
101	710	201	355	Utility filing fee	$355
102	320	206	160	Design filing fee	
104	490	207	245	Plant filing fee	
109	710	208	355	Reissue filing fee	
110	150	214	75	Provisional filing fee	
				Subtotal (1)	$355

2. CLAIMS

No. of claims as filed or after amendment			Claims Previously Paid		Extra claims		Fee from below		Fee Due
Total claims	20	-	20	=	0	x		=	$0
Ind. claims	2	-	3	=	0	x		=	$0
Multiple Dependent claims						x		=	

Large Fee Code	Entity Fee ($)	Small Fee Code	Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	80	202	40	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim
				Subtotal (2)

3. ADDITIONAL FEES

Large Fee Code	Entity Fee	Small Fee Code	Entity Fee	Fee Description	Fee Due	Large Fee Code	Entity Fee	Small Fee Code	Entity Fee	Fee Description	Fee Due
105	130	205	65	Surcharge - late filing fee or oath		127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification		147	2,520	147	2,520	Filing a request for reexamination	
115	110	215	55	Ext. for reply within first month		116	390	216	195	Ext. for reply within second month	
117	890	217	445	Ext. for reply within third month		118	1,390	218	695	Ext. for reply within fourth month	
128	1,890	228	945	Ext. for reply within fifth month		119	310	219	155	Notice of Appeal	
120	310	220	155	Filing brief in support of appeal		121	270	221	135	Request for oral hearing	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action		113	1840*	113	1840*	Requesting publication of SIR after Examiner action	
143	430	243	215	Design issue fee		144	580	244	290	Plant issue fee	
581	40	581	40	Recording patent assignment		Other fee (specify)					

* Reduced by Basic Filing Fee Paid

SUBTOTAL (3)

TOTAL AMOUNT TO BE CHARGED TO DEPOSIT ACCOUNT 50-0815 **($355.00)**

Submitted by (Typed Name)	Bret E. Field		**BOZICEVIC, FIELD & FRANCIS LLP**
Signature		Date 10-23-00	Reg. Number 37,620

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STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27 (b)) INDEPENDENT INVENTOR		
	Attorney Docket	YAMA-008
	First Named Inventor	Thomas Yamashita
	Application Number	N/A
	Filing Date	N/A
	Title	**MICROBIAL BLEND**

As a below named inventor, I hereby state that I qualify as an independent inventor as defined in 37 CFR 1.9 (c) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in: **MICROBIAL BLEND COMPOSITIONS AND METHODS FOR THEIR USE**

_X_ the specification filed herewith with title as listed above.
___ the application identified above.
___ the patent identified above.

I have not assigned, granted, conveyed, or licensed, and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

_X_ no such person, concern, or organization exists.
___ each such person, concern, or organization is listed below.

NAME: _____
ADDRESS: _____
___ Individual ___ Small Business Concern ___ Nonprofit Organization

Separate statements are required from each named person, concern or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

Thomas Yamashita NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Thomas Yamashita Signature of Inventor	Signature of Inventor	Signature of Inventor
10-12-2000 Date	Date	Date

"Express Mail" Mailing Label No. EL563390045US

Atty Docket No. YAMA-008

Date of Deposit 10-23-00

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Margaret Pierce
Typed or Printed Name of Person Mailing Paper or Fee

M. Pierce
Signature of Person Mailing Paper or Fee

PATENT APPLICATION

MICROBIAL BLEND COMPOSITIONS AND METHODS FOR THEIR USE

Bret E. Field
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Menlo Park, CA 94025

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MICROBIAL BLEND COMPOSITIONS AND METHODS FOR THEIR USE

INTRODUCTION

Field of the Invention

The field of this invention is agriculture

Background of the Invention

Agriculture is the science, art, and business of cultivating the soil, producing crops, raising livestock; and farming. With respect to cultivating the soil and producing crops, it is well known to add various fertilizing and other compositions to the soil and/or plant foliage in order to improve results. Agents that have been added to soil and/or plant tissues include microbial agents, which impart some beneficial property to the soil and/or plant to provided for desirable results.

There is continued interest in the development of new microbial formulations that are capable of providing beneficial results in agriculture and related fields.

Relevant Literature

U.S. Patents of interest include: 5,797,976; 5,696,094; 5,582,627; and 5,549,729. PCT applications of interest include: WO 00/13502 and WO 00/38513. See also: Mycorrhizae and Plant Health, F.L. Pfleger & R.G. Linderman, eds (1994) pp. 1-45; The Nature and Practice of Biological Control of Plant Pathogens, R.J. Cook & K.F. Baker (1983); and Microbial Ecology, Fundamentals and Applications. R.M. Atlas & R. Bartha, pp. 99-160

SUMMARY OF THE INVENTION

Microbial blend compositions and methods for their use are provided. The subject compositions are made up of a plurality of distinct microbial species that all share the following characteristics: (i) are antagonistic against a plurality of microbial pathogens; (ii) are non-pathogenic towards plants and animals; (iii) grow rapidly; (iv) are tolerant of high temperatures; and (iv) readily proliferate on a complex substrate. In many embodiments, the compositions further include a carrier, e.g., a liquid or solid carrier medium. In using the subject compositions, the compositions are applied to at least one of the soil and plant tissue, and in certain embodiments are applied in conjunction with a complex substrate. Also provided are methods of preparing the subject compositions.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Microbial blend compositions and methods for their use are provided. The subject compositions are made up of a plurality of distinct microbial species that all share the following characteristics: (i) are antagonistic against a plurality of microbial pathogens; (ii) are non-pathogenic towards plants and animals; (iii) grow rapidly; (iv) are tolerant of high temperatures; and (v) readily proliferate on a complex substrate. In many embodiments, the compositions further include a carrier, e.g., a liquid or solid carrier medium. In practicing the subject methods, the compositions are applied to at least one of the soil and plant tissue, and in certain embodiments are applied in conjunction with a complex substrate. Also provided are methods of preparing the subject compositions.

Before the subject invention is described further, it is to be understood that the invention is not limited to the particular embodiments of the invention described below, as variations of the particular embodiments may be made and still fall within the scope of the appended claims. It is also to be understood that the terminology employed is for the purpose of describing particular embodiments, and is not intended to be limiting. Instead, the scope of the present invention will be established by the appended claims.

In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

MICROBIAL BLEND COMPOSITIONS

As summarized above, the subject invention provides a composition that is made up of a plurality of distinct microbial species. By plurality is meant at least 2, and usually at least 5, where in many embodiments the number of different microbial species in the compositions may be as high as 10, 15 or higher. A feature of the subject compositions is that each of the constituent members of the plurality of microbial species has the following characteristics: (a) is antagonistic against a plurality of microbial pathogens; (b) is non-pathogenic towards plants and animals; (c) is tolerant of high temperatures; (d) grows rapidly; and (e) readily proliferates on a complex substrate. Each of these characteristics is now described in greater detail below.

By antagonistic against a plurality of microbial pathogens is meant that microbial species inhibits the growth of a plurality of known pathogenic microbial species, e.g., as determined in the assay described in the Experimental Section, infra. By plurality is meant at least 2, usually at least 5 and more usually at least 10. Specific known pathogenic microbial species against which the microbial species of the subject compositions preferably show antagonism include, but are not limited to:

(1)	*Verticillium dahliae*	*(7)*	*Monilochaetes infuscans*
(2)	*Fusarium solani*	*(8)*	*Rhizoctonia solani*
(3)	*Cylindrocarpon obtusisporum*	*(9)*	*Sclerotinia sclerotiorum*
(4)	*Pythium aphanidermatum*	*(10)*	*Sclerotinia minor*
(5)	*Phytophthora megasperma*	*(11)*	*Sclerotium rolfsii*
(6)	*Phymatotrichum omnivorum*	*(12)*	*Botrytis cinerea*

In certain preferred embodiments, the microbial species of the subject compositions show antagonism against at least 5 of the above pathogens, and more preferably against 10 of the above pathogens, and most preferably against all of the above pathogens. A particular

microbial species is considered to antagonistic against a microbial pathogen if it shows positive results in the assay described in greater detail in the Experimental Section, infra.

The microbial species of the subject compositions must also be non-pathogenic or non-toxic with respect to an array of plants and animals. Plants against which the microbial species of the subject compositions show substantially no or no toxicity include: Tomato Seedlings, Pepper Seedlings, Cucumber Seedlings, Radish Seedlings, and Grapevine Seedlings. Toxicity against these plants may be assessed using the assay described in the Experimental Section, infra. Animal species against which the particular microbial species of the subject compositions show substantially no or no toxicity as determined using the assay described in the experimental section, supra, include: mice and rabbits.

The microbial species of the subject compositions (microbial blends) must also be tolerant of high temperatures. By tolerant is meant that they are not inactivated or killed by exposure to high temperatures. As such, they are not inactivated or killed when exposed to temperatures up to 100, usually up to 120 and more usually up to 140 °F or higher.

In addition, microbial species of the subject compositions are rapid growers, i.e., they rapidly proliferate as determined using the assay described in the Experimental Section, infra. Using this growth assay, a species must meet or exceed 1 cm beyond the circle edge within twenty four hours to be a species suitable for inclusion in the subject compositions.

Additional preferred characteristics in many embodiments include tolerance to a wide range of pH conditions. As such, the species members of the subject compositions are preferably tolerant of pH conditions that range from 3.0 to 8.0. In addition, species present in the subject compositions preferably retain viability following a minimum of at least 100 days and usually at least 120 days in liquid suspension maintained at 70 ° F.

In addition to the above parameters, microbial species of the subject invention are those that provide for desired results in the greenhouse assays described in the experimental section, infra. In these assays, parameters that are evaluated are germination and stand %, completion of stand to production and/or harvest, production and quality, and post germination and post-stand infection.

In addition to the above requirements, all of the constituent members of the subject microbial blend compositions are ones that rapidly proliferate on a complex substrate. By complex substrate is meant a nutrient composition of matter that includes varied chain carbohydrates, amino acids, proteins, alcohols, organic acids, phenol derivatives and various cofactors. A representative complex substrate is provided in the experimental section, infra. Furthermore, complex substrates are disclosed in U.S. Patent Nos. 5,797,976; 5,696,094; 5,582,627; and 5,549,729; and published PCT application Nos. WO 00/13502 and WO 00/38513, the disclosures of which are herein incorporated by reference. A given microbial species is one that rapidly grows on a complex substrate if it grows on the substrate at a rate that is at least about 2 fold, usually at least about 5 fold and more usually at least about 10 fold faster than the specific pathogenic species disclosed above.

In certain embodiments, the constituent members of the subject microbial blend compositions are those that have been cultured or proliferated on a complex substrate, as described above and further detailed in the Experimental Section, infra.

The subject microbial blend compositions are further characterized in that they generally include at least 1 bacterial species and at least 1 fungal species. In many embodiments, the number of bacterial species in the composition is at least 5, while the number of fungal species is at least 2. In certain embodiments, the microbial species are naturally occurring species which are not genetically modified, i.e., have not been manipulated through recombinant DNA technology. Specific bacterial species of interest include, but are not limited to: *Bacillus subtilis; Bacillus thuringiensis; Bacillus cereus; Bacillus megaterium; Bacillus penetrans;* Arthrobacter paraffineus; and *Pseudomonas fluorescens.* Specific fungal species of interest include, but are not limited to: *Trichoderma viride, Trichoderma harzianum, Trichoderma polysporum, Trichoderma hamatum, Trichoderma koningii, Gliocladium virens, Gieocladium roseum, Gliocladium catenulatum, Penicillium oxalicum, Penicillium lilacinum, Penicillium nigricans, Penicillium chrysogenum, Penicillium frequentens,* and the like.

Preferably, the subject compositions are substantially, if not entirely, free of microbial species that do not meet the above described parameters. By substantially free is meant that less than 1%, usually less that 0.5% and more usually less than 0.1 % of the

total number of microbial species in the composition do not meet the above described parameters.

The subject microbial blend compositions may include a carrier medium, which carrier medium may be a liquid or solid. Liquid carrier mediums of interest include aqueous mediums, e.g., water, which may or may not include additional components, e.g., which may or may not include additional components, e.g., glycerin, alcohol(s), polymers, organic acid(s), microbial by-products such as amino acids, various organic acids, complex carbohydrates, macronutrients, micronutrients, vitamins & cofactors, sterols, proteins, gums (e.g. guar gum, xanthan gum), liquid fertilizers, liquid substrates, e.g., as found in co-pending patent application serial no. 9/222,459; and the like. When present in a liquid medium, the total number of microbial species in the medium is generally at least about 1 x 10^5cfu/ml, usually at least about 1 x 10^9 cfu/ml and more usually at least about 1 x 10^{12}cfu/ml. Carrier materials of interest also include solid media, e.g., inactivated seed, viable seed, prilled fertilizer, pelletted fertilizer, potting soil, compost, soybean or related meal, greenwaste and related organic waste, manure, fruit culls, talcum, dry mineral preparations, etc. and the like. When combined with a solid medium, the total number of microbial species in the overall composition generally ranges from about 1 x 10^3 to 1 x 10^{14}, usually from about 1 x 10^4 to 1 x 10^{12} and more usually from about 1 x 10^5 to 1 x 10^9.

METHODS OF USE

In practicing the subject methods, the subject compositions are applied to at least one of: the plant, a portion thereof and soil associated therewith. As such, the composition is, in many embodiments, applied to foliage of the plant, e.g. either the entire part of the plant which is above the soil level or a portion thereof, e.g. fruit, leaves, etc. In other embodiments, the composition is applied to soil associated with the plant, i.e. soil proximal to the plant in which the plant is growing, i.e. soil that is contacted by the roots of the plant or from which the plant's roots ultimately obtain nutrients and/or water.

A variety of different application protocols may be employed to apply the subject compositions, where the particular protocol employed depends, at least in part, on whether the particular compositions is a solid or liquid composition. Where the

compositions is a liquid, in certain embodiments, the liquid composition is contacted with the soil. By contact is meant that the composition is introduced into the soil. As such, contact can include spraying so that the composition soaks into the soil, injecting the composition into the soil, flooding the soil with the composition, and the like. In yet other embodiments, the composition is contacted with at least a portion of the foliage of the plant. By contact in this context is meant that the composition is placed on the surface of the foliage of the plant(s) to be treated, where the term "foliage" is used broadly to encompass not only the leaves of the plant, but every other part of the plant that is not underground, i.e., below the soil surface, such that the term "foliage" includes leaves, stems, flowers, fruit, etc. Contact may be by any convenient method, including spraying, applying etc.

Depending on the particular protocol being performed and the desired outcome, as well as the nature of the composition, the environmental conditions and any other factors, the composition may be applied more than once over a given period of time. As such, the composition may be applied daily, weekly, every two weeks, monthly etc.

In many embodiments of the subject invention, the liquid compositions described above are applied or delivered in combination with an aqueous delivery vehicle. The aqueous delivery vehicle may be pure water, e.g. tap water, or an aqueous compositions that includes a carbohydrate source and other components. Of interest in many embodiments as aqueous delivery vehicles are those aqueous compositions described in copending application serial nos. 09/149,930 and 09/222,459, as well as those described in U.S. Patent Nos. 5,797,976; 5,696,094; 5,582,627; and 5,549,729; and published PCT application Nos. WO 00/13502 and WO 00/38513, the disclosures of which are herein incorporated by reference (and specifically, the complex substrates disclosed in these patents, applications and publications); the disclosures of which are herein incorporated by reference. When delivered in combination of with an aqueous delivery vehicle, the ratio of the liquid microbial blend composition to vehicle typically ranges from about 4 oz microbes with 27,000 gal vehicle to 10 gal microbes with 27,000 gal vehicle, usually from about 1 qt microbes with 27,000 gal vehicle to 5 gal microbes with 27,000 gal vehicle and more usually from about 2 qt microbes with 27,000 gal vehicle to 2.5 gal microbes with 27,000 gal vehicle.

The rate at which the subject liquid compositions are applied to the plants may vary depending on the particular nature of the composition and the method by which it is applied, so long as a sufficient amount of the composition is applied to obtain the desired results. In many embodiments where the liquid compositions are applied to the soil, the rate of application ranges from about 4 oz to 5 gal, usually from about 1 qt to 2.5 gal and more usually from about 2 qt to 1 gal/ acre. Alternatively, where the liquid compositions are applied to plant tissue, e.g., foliage, they are generally applied at a rate of about 4 oz to 10 gal, usually from about 1 qt to 5 gal and more usually from about 2 qt to 2.5 gal liquid composition per 100 gallons liquid carrier, e.g., water with which the composition is blended immediately prior to application.

In those embodiments where the composition is a dry composition, e.g., a blend coated onto a dry carrier, such as inactivated seed, etc., the composition is, in many embodiments, applied to the soil. Application may take various formats, including broadcast onto the soil top, e.g., 4 to 10 inches, or to the soil surface. The dry composition may also be blended with seeded species during drilling. Other applications protocols may be employed, as are convenient. In many embodiments of using the dry compositions, the compositions are applied at a rate of 8 oz to 500 lbs, usually from about 2 lbs to 400 lbs and more usually from about 15 lbs to 200 lbs/acre.

UTILITY

The subject methods and compositions find use in a variety of different applications. For example, the subject compositions and methods may be used for: 1) Antagonism of soil-borne pathogens, e.g., as evidenced by a 10%-100% reduction in inoculum levels as compared to a control; 2) Antagonism of soil-overwintering pathogens, e.g. as evidenced by a 10%-100% reduction as compared to a control; 3) Increased release of tied-up minerals, as evidenced by a 25%-500% increase as compared to a control; 4) Antagonism of pests and nematodes, as evidenced by a 10%-100% reduction as compared to a control; 5) Increased water infiltration rates as evidenced by a 25%-800% increase as compared to a control; 6) Increased water-holding capacity of soil as evidenced by a 5%-50% increase as compared to a control; 7) Aerial pathogen antagonism, as evidenced by a 10%-100% reduction as compared to a control; 8) Aerial

pest antagonism, as evidenced by a 10%-100% reduction as compared to a control; 9) Reduced freeze hypersensitivity, as evidenced by a 10%-100% reduction as compared to a control; 10) Extended shelf life of fruits & vegetables as evidenced by a 10%-100% increase as compared to a control; 11) Antagonism of insect pests as evidenced by a 10%-100% reduction as compared to a control; 12) Antagonism of soil-borne pathogens as evidenced by a 10%-100% reduction as compared to a control; 13) Antagonism of soil-overwintering pathogens as evidenced by a 10%-100% reduction as compared to control; 14) Increased release of tied-up minerals as evidenced by a 10%-100% increase as compared to a control; 15) Antagonism of nematode pests as evidenced by a 10%-100% reduction as compared to a control; etc.

METHODS OF MAKING

Also provided are methods of making the subject formulations. A representative manufacturing method is provided in the experimental section, infra. Briefly, to prepare the subject microbial blend compositions, the microbes to be included in the composition are first identified. This identification step may include using microbes that are known to meet the above listed criteria or screening candidate microbes to determine whether they possess the desired criteria. Once the microbe constituents are identified, they are then matured or grown in culture, preferably separately and on a complex substrate, as described above. The separate grown and matured microbial cultures are then combined to produce the final microbial blend compositions, which may then be combined with a carrier, as desired.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

I. Identification of Microbes

The beneficial, antagonistic strains are isolated from California farm land. They are natural, non-engineered isolates. Candidate isolates are put through a rigorous testing scheme before being considered for use in the finished suspension for commercial use:

A. Pathogen Antagonistic Assay:

1. Overview

Candidate agents are tested on "Challenge Plates" on which the petri dish-containing media is inoculated with 2 discs of one of 12 common soil-inhabiting pathogenic species –

(1) Verticillium dahliae	*(7) Monilochaetes infuscans*
(2) Fusarium solani	*(8) Rhizoctonia solani*
(3) Cylindrocarpon obtusisporum	*(9) Sclerotinia sclerotiorum*
(4) Pythium aphanidermatum	*(10) Sclerotinia minor*
(5) Phytophthora megasperma	*(11) Sclerotium rolfsii*
(6) Phymatotrichum omnivorum	*(12) Botrytis cinerea*

In this assay, a candidate beneficial antagonist must show aggressiveness against all 12 pathogenic species. A single streak of the candidate beneficial microbe is cultured between the 2 discs of the pathogen. The zone of inhibition to inward progressive growth of the pathogen manifests, in part, the potential antagonistic capabilities of the candidate.

2. Details

Method of culturing microbe species candidates:

a) Bacterial candidates are cultured on nutrient agar (Bacto Nutrient Agar, DIFCO Laboratories, Detroit, MI) as a standard agar medium (31 grams per liter of media)
b) Fungal candidates are cultured on potato dextrose agar (DIFCO Laboratories, Detroit, MI) as a standard agar medium (39 grams per liter of media)
c) Actinomycete candidates are cultured onto the following agar medium:

Ingredient	*Amount/Liter*
Beef extract	1 gr
Yeast extract	1 gr
Tryptose	2 gr
Glucose	10 gr
Ferrous sulfate	trace
Agar	15 gr
Water	1,000 ml

d) Candidate isolates are cultured onto appropriate media @ 25 deg C. Fungal species that require light for sporulation are cultured in the light. Otherwise, all other cultures are incubated in the dark.

e) Thriving cultures of the pathogen are also cultured on appropriate media. A 5 mm diameter disc is removed from the test agar plate and replaced with a matching disc from the pathogen culture. At the same time a 5 mm wide strip of candidate antagonist is streaked in the middle of the plate, exactly between the 2 pathogen discs. These will be referred to as "Challenge Plates". Two matching control plates are also set up at the same time: (a) With pathogen discs only and (b) With antagonist streak only.

f) The challenge and control plates are incubated in the dark at 25 deg C and examined at 24 hour intervals.

g) Criteria for accepting a viable antagonist candidate are as follows:
   (1) The antagonist must either match or exceed the rate of growth of the pathogen
   (2) If "zones of inhibition" are manifested, the zone of inhibition must exceed 25% impedance of the growth indicated on the pathogen control plate
   (3) Concomitantly, the growth of the antagonist must not be impeded by more than 25% of the growth observed on the antagonist candidate control plate
   (4) Antagonism must be observed within 48 hours
   (5) More than 50% of the pathogen growth must be impeded by the candidate antagonist

B. Identification of Candidates and Evaluation of Plant/Animal Toxicity:

1. Overview

Candidates that pass the pathogen antagonism test are then identified to the species level, using any convenient protocol. Part of the reason for speciation is to clearly identify any possible animal or plant pathogens. Species that might be suspected of being potential animal or plant pathogens are tested as follows –

a. Plant Pathogen Screening : Test Plants ~Tomato Seedlings, Pepper Seedlings, Cucumber Seedlings, Radish Seedling, Grapevine Seedling.

Tests: Suspension Hypodermic Needle Injection Into Vascular Tissue

Suspension Spray + Humid Incubation

b. Animal Pathogen Screening: Test Animals ~ Rabbit & Mice

Tests: Suspension Hypodermic Needle Subcutaneous Injection

Suspension Spray Exposure / Lung Inhalation

2. Details

Method of Pathogen Antagonism Screening with Indicator Plants ~

Pots with various types of soil are prepared:

a) Sterilized control
b) Inoculated with appropriate disease-causing levels of pathogens –

Pathogen	***Approx Inoculum per Gram Soil***
Verticillium dahliae	200+ cfu
Fusarium solani	400+ cfu
Rhizoctonia solani	30+ cfu
Pythium aphanidermatum	300+ cfu
Phytophthora megasperma	50+ cfu
Phymatotrichum omnivorum	100+ cfu
Monilochaetes infuscans	400+ cfu
Sclerotinia sclerotiorum	5+ cfu
S. minor	25+ cfu
Sclerotium rolfsii	10+ cfu
Botrytis cinerea	400+ cfu

Forty eight hours after pathogen introduction, contaminated and control soils (250 cc) are drenched with a suspension of the candidate antagonist:

c) Bacteria are drenched at 50 ml of suspension @ approximately 1 x 10 (12^{th}) cfu per ml + 5 ml liquid substrate (Pending USA Patent application no. 9/222,459, the disclosure of which is herein incorporated by reference)
d) Fungi are drenched at 50 ml of suspension @ approximately 1 x 10 (9^{th}) cfu per ml + 5 ml of liquid substrate (above)
e) Actinomycetes are drenched at 50 ml of suspension @ approximately 1 x 10 (10^{th}) per ml + 5 ml of liquid substrate (above)
f) A control series is run with just 5 ml/250 cc soil of substrate alone

The pathogen + antagonist and control pots are allowed to incubate for 2 weeks, keeping the soil reasonably moist (~80% field capacity) throughout the 2 weeks, which allows for microbe activity.

At the end of 2 week's incubation, the pots are seeded with appropriate indicator plants.

Criteria utilized for the various pathogens are:

Pathogen	***Criteria for Antagonism***	***Passing Grade***
Verticillium dahliae	Vascular wilt @ or after bloom	< 10% of CK
Fusarium solani	Root & stem rot development	< 10% of CK
Cylindrocarpon obtusisporum	Vascular wilt @ or after bloom	< 10% of CK
Pythium aphanidermatum	Germination & stand %	> 90% of CK
Phytophthhora megasperma	Germination & stand %	> 90% of CK
Phymatotrichum omnivorum	Germination & stand %	> 90% of CK
Monilochaetes infuscans	Reisolation and titer of the pathogen	< 10% of CK
Rhizoctonia solani	Root & stem rot; germ & stand %	< 10% of CK
Sclerotinia sclerotiorum	Reisolation of sclerotia and viability	< 10% of CK
S. minor	As for *S. sclerotiorum*	< 10% of CK
Sclerotium rolfsii	Reisolation of sclerotia and viability	< 10% of CK
Botrytis cinerea	Reisolation and titer of pathogen	< 10% of CK

Candidate antagonists which pass the plate and greenhouse bioassay are cultured onto appropriate agar plates and incubated @ 25 deg C for 48-96 hours.

Rabbits and mice are exposed as follows:

a) Lung exposure - a liquid suspension of ~1 x 10 (6^{th}) cfu/ml is sprayed via an aerosol mist while the animal is placed within an air-tight enclosure. The same exposure is made to control animals but with sterile distilled water (CK).
b) Intravenous injection – a liquid suspension of ~1 x 10 (6^{th}) cfu/ml is injected behind the neck (~100 mcl). A control exposure utilizes 100 mcl of sterile distilled water.
c) Oral ingestion - a liquid suspension of ~1 x 10 (6^{th}) cfu/ml is sprayed onto food and drinking water replaced with 10 ml/100 ml water suspension. The control treatment merely covers the use of sterile distilled water sprayed over solid food.

For the lung and intravenous exposures, animals are allowed to resume their normal activities and observed for 2 months. Oral ingestion is allowed to continue for 1 week before normal activities are resumed and observed for 2 months.

Criteria for evaluations are as follows:

d) Coughing or respiratory difficulties
e) Lesions or infections
f) Loss of weight or appetite
g) Mortality

All tests have a 0% tolerance for a discrepancy in any of the above categories

C. Additional Screening Assays:

1. Overview

Candidate, beneficial microbes are further characterized based on alternative characteristics ~

a) Maximum temperature tolerance (preferably tolerant to at least 140 deg F); this tends to select spore-forming bacteria, actinomycetes and resting stage spore-forming fungi
b) Tolerant of pH range from 3.0 – 8.0
c) Rapid growth rate (when a central, circular inoculum is placed on media, the candidate must meet or exceed 1 cm beyond the circle edge within 24 hrs)
d) Retention of viability following a minimum of 120 days in liquid suspension @ 70 deg F

2. Details

Test	*Method & Evaluation Criteria*
a. Max Temp	- Candidate antagonists cultured onto appropriate agar media - 96 hr cultures exposed to: 140 deg F for 96 hours - Reisolation and % viability determined - A 90%+ recovery is required to pass this test
b. pH Tolerance	- Candidate antagonist suspensions set @ pH 3, 5 & 8 (1 x 10-12th) - Exposed for 96 hours @ 25 deg C - Reisolation and examination of titer - A 90%+ recovery is required to pass this test
c. Growth Rate	- 5 mm discs of candidates are placed onto appropriate media (1 disc in the middle and 1 disc within each quadrant) - All are incubated at 25 deg C in the dark except for species that require light (e.g *Trichoderma spp.*) - Organisms must meet the following criteria:

a) Fungi - Fill the plate in 72 hours
b) Bact - Fill 60% of the plate within 96 hours
c) Act - Fill 60% of the plate within 96 hours

d. Viability
- Candidates are grown and matured on appropriate agar media @ 25 deg C for 120 hours.
- Organisms are washed from the plates with a light saline solution (Ringer's Solution) and made up to 1 x 10 (12^{th}) concentration.
- The containers are labeled and placed in dark rooms set @ 25 deg C for 120 days
- After exposure, the titer of viable organisms is tested
- 80%+ viability is required to pass the test

D. Growth Enhancement Assays:

The safe and efficacious, beneficial, pathogen antagonistic microbes identified in the above assays are then further tested under simulated field conditions utilizing model, potted plant studies –

e) Tomato Seedlings + (1) *Pythium aphanidermatum*
+ (2) *Rhizoctonia solani*
+ (3) *Verticillium dahliae*
+ (4) *Fusarium oxysporum*

f) Lettuce Seedlings + (1) *Pythium aphanidermatum*
+ (2) *Sclerotinia sclerotiorum*

c) Pepper Seedlings + (1) *Phytophthora parasitica*
+ (2) *Rhizoctonia solani*
+ (3) *Sclerotium rolfsii*
+ (4) *Fusarium solani*

g) Parameters Examined ~
i. Germination and stand %
ii. Completion of stand to production and/or harvest
iii. Production and quality
iv. Post-germination and post-stand infections

II. Microbial Blend Preparation:

The beneficial, pathogenic antagonistic microbial candidates passing all tests described above are then mass produced individually in pure culture, allowed to mature, then blended together for the final product suspension. The following aqueous medium is employed for culture:

Material	*Targeted Ingredients*	*Rate/100 Gal Mix*
Molasses	simple & complex sugars, cofactors, proteins	2 gal
Ca Lignosulfonate	phenolic derivatives, various acids, complex sugars	2 qt
Amino Acids	aliphatic, acidic, basic and other amino acids	1 gal
Gallic Acid	phenolic acid	1 lb
Yeast Extract	cofactors, vitamins	10 lb
Tap Water	-	~96 gal

Note:

(1) The blend is ozonated for 6-12 hours to remove contaminants, then allowed to dissipate residual ozone for 2 hours with sterile air bubbling before a gallon of 48-hour liquid starter culture is added.

(2) The large inoculum of starter culture is further assurance to avoid contamination.

(3) The culture is allowed to reach maturity for 72-120 hours following inoculation.

(4) Maturity is gauged by the final pH of the suspension. Most cultures are mature when the pH drops close to 4.0-4.5

(5) Cultures are then blended in equal volumes and homogenized in a stainless steel mixing vat.

(6) The natural, organic acid by-products induced to production assist in maintaining a quiescent state of the microbes.

(7) The mixed and defined suspension is containerized and stored between 36-70 deg F.

Note:

[1] Certain species of fungi (e.g. *Trichoderma viride*, *Gliocladium virens*) are cultured on cooked grain.

[2] The grain is first boiled in the media described above, then sterilized in an autoclave (120 psi, 240 deg F)

[3] The sterilized, media-impregnated grain is then cooled and inoculated with pure spore suspensions of the required fungus, covered to prevent contamination and incubated between 70-80 deg F for 1 week.

[4] Spores are harvested by submersing the grain culture (covered with spores) in Ringer's Salt Solution into which silicone surfactant is added to make a 100-200 ppm surfactant solution.

[5] The spore suspension is standardized to 1-10 billion per ml and the suspension added to the mixing vat in step 5 above (10 gal/100 gal mix).

III. Representative Specific Compositions and Methods of Use

A. Specific Formulations

Material	***Constitution***	***Volume / Gal***	***Final Product Appox Titer***

Iota	*Bacillus subtilis 201*	16 oz	~ 5 x 10 (11^{th})
	Bacillus subtilis 202	16 oz	~ 5 x 10 (11^{th})
	Comomonas acidovorans	16 oz	~ 5 x 10 (11^{th})
	Curtobacterium sp.	16 oz	~ 5 x 10 (11^{th})
	Pseudomonas fluorescens 301	16 oz	~ 5 x 10 (11^{th})
	Bacillus thuringiensis 102	16 oz	~ 5 x 10 (11^{th})
	Trichoderma viride 401	32 oz	~ 5 x 10 (9^{th})

Iota (+)	*B. subtilis 201*	16 oz	~ 5 x 10 (11^{th})
	B. subtilis 202	16 oz	~ 5 x 10 (11^{th})
	B. thuringiensis 101	21 oz	~ 7 x 10 (11^{th})
	B. thuringiensis 102	21 oz	~ 7 x 10 (11^{th})
	B. thuringiensis 103	21 oz	~ 7 x 10 (11^{th})
	Trichoderma viride 401	32 oz	~ 5 x 10 (9^{th})

Asunder	Heat inactivated corn seed	50 lbs	~ 2 x 10 (11^{th})
	Iota (+) suspension	250 ml	
	Spreader Sticker	2 ml	

B. Benefits

Product	***Benefits***	***Measure of Benefit***
Soil:		
Iota	1) Antagonism of soil-borne pathogens	1) 10%-100% reduction in inoculum levels
	2) Antagonism of soil-overwintering pathogens	2) 10%-100% reduction
	3) Increased release of tied-up minerals	3) 25%-500% increase
	4) Antagonism of pests and nematodes	4) 10%-100% reduction
	5) Increased water infiltration rates	5) 25%-800% increase
	6) Increased water-holding capacity of soil	6) 5%-50% increase
Iota	1) Aerial pathogen antagonism	1) 10%-100% reduction
Foliar:	2) Aerial pest antagonism	2) 10%-100% reduction
	3) Reduced freeze hypersensitivity	3) 10%-100% reduction
	4) Extended shelf life of fruits & vegetables	4) 10%-100% increase
Asunder:		
	1) Antagonism of insect pests	1) 10%-100% reduction
	2) Antagonism of soil-borne pathogens	2) 10%-100% reduction
	3) Antagonism of soil-overwintering pathogens	3) 10%-100% reduction
	4) Increased release of tied-up minerals	4) 10%-100% increase
	5) Antagonism of nematode pests	5) 10%-100% reduction

C. Additional Formulation

Ingredient	***Amount per Lb.***
Heat-Inactivated Corn Seed	-
Bacillus thuringiensis 101	1.2 ml

Bacillus thuringiensis 102	1.2 ml
Bacillus thuringiensis 103	1.2 ml
Bacillus subtilis 201	1.2 ml

Note: Each liquid culture of microorganisms contains 1 x 10 (9^{th}) – 1 x 10 (14^{th}) cfu/ml

IV. Characterization Assays:

A. Rhizoctonia solani *suppression* –

Sandy loam soil in 6" diameter clay pots sterilized by autoclaving (120 psi, 110 deg C, 1 hr)

Pathogen treatments -

Inoculated with 10 sclerotia per gram of soil (Control)

Inoculated with 10 sclerotia per gram of soil (Treatment)

Antagonist treatments (per 250 cc soil) -

Drenched with 50 ml sterile water (Control)

Drenched with 50 ml of Iota suspension (1 x 10 (12^{th} cfu/ml) + 5 ml liquid substrate (U.S. Patent application no. 9/222,459, the disclosure of which is herein incorporated by reference)

Allowed to incubate 14 days:

25 deg C

80% field capacity wetness

~16 hours light + 8 hours darkness

After 14 days incubation, 10 control and 10 treatment pots planted with green bean (*Phaseolus vulgaris*)

After 21 days, inspected for root and stem lesions and rated on a 1-10 scale with 10 representing maximum disease

***Rhizoctonia solani*: Antagonism in the soil with Iota**

Treatment	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***Total***	***Mean***
Control	9	8	10	9	9	7	10	8	9	9	88	8.8 a
Iota	1	2	1	1	1	1	1	2	1	1	12	1.2 b

B. *Verticillium dahliae* suppression -

1. Sandy loam soil in 6" diameter clay pots sterilized as for *R. solani*
2. Pathogen treatments –
   a) Inoculated with 200 microsclerotia per gram of soil (Control)
   b) Inoculated with 200 microsclerotia per gram of soil (Treatment)
3. Antagonist treatments (per 250 cc soil) –
   a) Drenched with 50 ml sterile water (Control)

Drenched with 50 ml of Iota suspension (1 x 10 (12^{th}) cfu/ml) + 5 ml liquid substrate (U.S. Patent

application no. 9/222,459, the disclosure of which is herein incorporated by reference)

b) Allow to incubate 21 days:
   (1) 25 deg C
   (2) 80% field capacity wetness
   (3) ~16 hours light + 8 hours darkness

4. After 21 days incubation, 10 control and 10 treatment pots planted with green bean (*Phaseolus vulgaris*)
5. Plants allowed to grow past bloom and into fruit set before evaluation of disease. Plants were evaluated for visible wilt symptoms and given a 1-10 rating with 10 representing maximum disease.

***Verticillium dahliae*: Antagonism in the soil with Iota**

Treatment	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***Total***	***Mean***
Control	10	10	9	10	10	10	9	10	9	10	97	9.7 a
Iota	1	1	1	1	1	1	1	1	1	1	10	1.0 b

C. **Sclerotium rolfsii *suppression* –**

Identical soil preparation as per *R. solani*
Pathogen treatments same as per *R. solani* but with *S. rolfsii*
Antagonist treatment same as per *R. solani* but incubation time increased to 21 days
After 21 days incubation, 10 control and 10 treatment pots planted with green bean (*Phaseolus vulgaris*)
Plants allowed to grow 10 days past fruit set before evaluation for crown rot. Rated on a 1-10 scale with 10 representing maximum disease.

***Sclerotium rolfsii*: Antagonism in the soil with Iota**

Treatment	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***Total***	***Mean***
Control	10	10	10	10	10	9	10	10	10	10	99	9.9 a
Iota	1	1	1	1	1	1	1	1	1	1	10	1.0 b

D. Phytophthora cactorum *suppression* –

Soil prepared as per *R. solani*
Pathogen treatments –
   Inoculated with 40 cfu per ml (Control)
   Inoculated with 40 cfu per ml (Treatment)
Antagonist treatments (per 250 cc soil) –
   Drenched with sterile water

Drenched with 50 ml Iota suspension (1 x 10 (12^{th}) cfu/ml) + 5 ml liquid substrate (U.S. Patent application no. 9/222,459, the disclosure of which is herein incorporated by reference)
Allowed to incubate 14 days:
25 deg C
80% field capacity wetness
~16 hours light + 8 hours darkness
After 14 das incubation, 10 control and 10 treatment pots planted with green bean (*Phaseolus vulgaris*)
Plants allowed to grow for 21 days before evaluation for crown and rot rot. Rated on a 1-10 scale with 10 representing maximum disease.

***Phytophthora cactorum*: Antagonism in the soil with Iota**

Treatment	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***Total***	***Mean***
Control	10	10	10	10	10	10	10	10	10	10	100	10 a
Iota	2	2	2	1	4	2	3	1	2	2	21	2.1 b

E. Botrytis cinerea suppression –
Soils prepared as per *R. solani*
Pathogen treatments –
Inoculated with ~400 cfu per gram of soil (Control)
Inoculated with ~400 cfu per gram of soil (Treatment)
Antagonist treatments (per 250 cc soil) –
Drenched with 50 ml sterile water (Control)
Drenched with 50 ml of Iota suspension (1 x 10 (12^{th}) cfu/ml) + 5 ml liquid substrate (U.S. Patent application no. 9/222,459, the disclosure of which is herein incorporated by reference)
Allowed to incubate 14 days:
25 deg C
80% field capacity wetness
~16 hours light + 8 hours darkness
After 14 days incubation, 10 control and 10 treatment pots isolated for *Botrytis cinerea* inoculum levels
Evaluation based on cfu's recovered based on a 400 cfu/ml inoculation. Rating was based on a 1-10 scale with 10 representing maximum recovery of the pathogen.

***Botrytis cinerea:* Antagonism in the soil with Iota**

Treatment	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***Total***	***Mean***
Control	10	10	10	10	10	10	10	10	10	10	100	10 a

Iota	3	2	2	1	1	3	3	2	3	3	23	2.3 b

F. Sclerotinia sclerotiorum *suppression* –

Soil preparation as per *R. solani*

Pathogen treatments –

Inoculated with 5 sclerotia per gram of soil and placed in nylon sock and buried in the potting soil (Control)

Inoculated with 5 sclerotia per gram of soil as above (Treatment)

Antagonist treatments (per 250 cc soil) –

Drenched with 50 ml sterile water (Control)

Drenched with 50 ml of Iota suspension (1 x 10 (12^{th}) cfu/ml) + 5 ml liquid substrate (U.S. Patent application no. 9/222,459, the disclosure of which is herein incorporated by reference)

Allowed to incubate 21 days:

25 deg C

80% field capacity wetness

~16 hours light + 8 hours darkness

After 21 days incubation, 10 control and 10 treatment pots examined for sclerotia viability.

Viability based on a 1-10 rating with 10 representing maximum viability

***Sclerotinia sclerotiorum*: Antagonism in the soil with Iota**

Treatment	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***Total***	***Mean***
Control	10	10	10	10	10	10	10	10	10	10	100	10 a
Iota	1	1	1	1	1	1	1	1	1	1	10	1 b

The above discussion and results demonstrate that the subject microbial blend compositions provide for significant benefits in the field of agriculture, where use of the subject compositions in accordance with the subject methods provides for significantly improved results. As such, the subject invention represents a significant contribution to the art.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of skill in the art that many changes and modifications can be made thereto without departing from the spirit and scope of the appended claims.

WHAT IS CLAIMED IS:

1. A composition comprising a plurality of distinct microbial species, wherein each constituent member of said plurality is:

(a) antagonistic against a plurality of microbial pathogens;

(b) non-pathogenic towards plants and animals;

(c) is tolerant of high temperatures;

(d) grows rapidly; and

(e) proliferates on a complex substrate.

2. The composition according to Claim 1, wherein said plurality comprises at least one bacterial species and at least one fungal species.

3. The composition according to Claim 2, wherein said plurality comprises at least 5 distinct microbial species.

4. The composition according to Claim 3, wherein said plurality comprises at least 5 bacterial species.

5. The composition according to Claim 3, wherein said plurality comprises at least 2 fungal species.

6. The composition according to Claim 1, wherein said composition comprises a carrier.

7. The composition according to Claim 6, wherein said carrier is a liquid.

8. The composition according to Claim 6, wherein said carrier is a solid.

9. The composition according to Claim 1, wherein said plurality of microbial species has been proliferated on a complex substrate.

10. A composition comprising:

(a) a plurality of distinct microbial species made up of at least 5 different bacterial species and at least 2 different fungal species, wherein each constituent member of said plurality is:

(i) antagonistic against a plurality of microbial pathogens;

(ii) non-pathogenic towards plants and animals;

(iii) is tolerant of high temperatures;

(iv) grows rapidly; and

(v) proliferates on a complex substrate; and

(b) a carrier.

11. The composition according to Claim 10, wherein said carrier is a liquid.

12. The composition according to Claim 10, wherein said carrier is a solid.

13. In an agricultural method, the improvement comprising:
applying to at least one of soil or plant tissue a composition according to Claim 1.

14. A method of producing a composition according to Claim 1, said method comprising:

(a) identifying a plurality of microbial species that are:

(i) antagonistic against a plurality of microbial pathogens;

(ii) non-pathogenic towards plants and animals;

(iii) tolerant of high temperatures;

(iv) grows rapidly; and

(v) proliferates on a complex substrate; and

(b) combining said plurality to produce said composition.

15. The method according to Claim 14, wherein said method further comprises separately proliferating each species prior to said combining.

16. The method according to Claim 15, wherein said proliferating occurs in the presence of a complex substrate.

17. The method according to Claim 15, wherein said method further comprises combining said composition with a carrier.

18. The method according to Claim 17, wherein said carrier is a fluid.

19. The method according to Claim 17, wherein said carrier is a solid.

20. The method according to Claim 14, wherein said identifying comprises subjecting a candidate microbial species to a series of assays which identify whether the species has all of said (i)-(v) characteristics.

MICROBIAL BLEND COMPOSITIONS AND METHODS FOR THEIR USE

ABSTRACT OF THE DISCLOSURE

Microbial blend compositions and method for their use are provided. The subject compositions comprise a plurality of distinct microbial species that all share the following characteristics: (i) are antagonistic against a plurality of microbial pathogens; (ii) are non-pathogenic towards plants and animals; (iii) are tolerant of high temperatures; (iv) grow rapidly; and (v) proliferate on a complex substrate. In many embodiments, the compositions further include a carrier, e.g., a liquid or solid carrier medium. In practicing the subject methods, the compositions are applied to at least one of soil and plant tissue, and in certain embodiments are applied in conjunction with a complex substrate. Also provided are methods of preparing the subject compositions.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)		
	Attorney Docket Number	YAMA-008
	First Named Inventor	Thomas Yamashita
	Application Number	N/A
	Filing Date	Herewith
	Group Art Unit	N/A
	Examiner Name	N/A

X Declaration Submitted with Initial Filing OR ___ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e)) required)

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MICROBIAL BLEND COMPOSITIONS AND METHODS FOR THEIR USE

the specification of which:

X is attached hereto

OR

___ was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined by 37 CFR 1.56.

Insofar as the subject matter of each of the claims of this application are not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designating at least one country other than the United States of America, listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) having a filing date before that of the application(s) of which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application(s) designating the United States of America, listed below.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

Given Name (first and middle [if any])	Family Name or Surname
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